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IMPROVED METHOD FOR THE ANALYSIS OF SMALL AMOUNTS OF ESSENTIAL OILS BY MICRODISTILLATION FOLLOWED BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A method for the analysis of small amounts of essential oils by distillation in a microversion of a modified Marcusson apparatus followed by capillary gas chromatography is described. The analysis requires only very small amounts of plant material (0.2–3 g) and takes no more than 2 h, including sample preparation, distillation and capillary gas chromatography. The results of macroscale analysis by classical techniques were identical with those obtained with the microapparatus described in this paper. Some applications are given to illustrate this technique.

INTRODUCTION

Distillation systems for very small amounts of plant material and subsequent adequate analytical techniques must be used in several aspects of the study of essential oil composition. The possibility of analysing very small amounts of sample can in fact be fundamental in both chemotaxonomy and control analysis.

The analytical problem can be solved, in part, by capillary gas chromatography (GC) because of its high sensitivity and resolving power for such complex mixtures. Preparation techniques for micro amounts of essential oils need further improvement, although very important contributions have already been made to this problem.

In 1981, Godefroot *et al.*[1] described a method for quantitative essential oil analysis by a combined steam distillation and extraction. Their micro-scale apparatus collects all the volatile material in only 1 ml of dichloromethane, containing an internal standard. Amounts of 1–15 g of plant material are employed, and no further enrichment by evaporation is required.

Gabri and Chialva[2,5] developed a method for the GC headspace evaluation of herbs and drugs. The procedure consists in milling a weighed sample (0.1–0.5 g) of a herb in a glass mill such as a coffee grinder, which is modified so that a special gas-tight valve allows direct sampling. Some very volatile compounds that cannot be detected in essential oils were found in the headspace; these components may be important constituents of plant flavours.

In this paper, a new apparatus for microdistillation is presented. The apparatus

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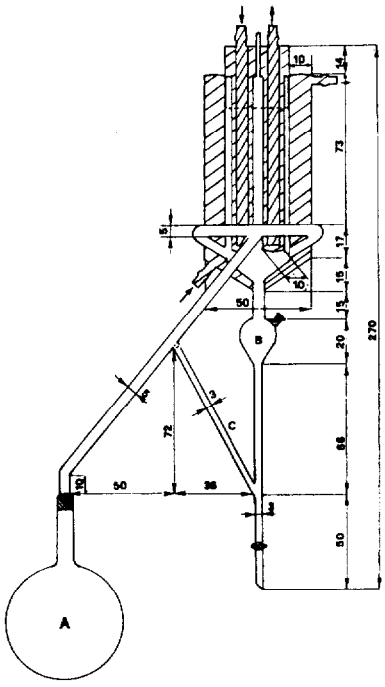


Fig. 1. Microdistillation apparatus. Dimensions in millimetres.

(Fig. 1) works on the same principle as the modified Marcusson apparatus[3], which has been used in several laboratories to obtain essential oils with good results. Its dimensions were reduced as far as pratically possible, in order to reduce to a minimum the amount of plant material required and to obtain the highest possible efficiency of heat exchange on the smallest possible surface, thus avoiding loss of volatiles. The apparatus was calibrated with a suitable standard mixture.

This method, which is applicable to very small amounts of plant material, is reliable and faster than classical techniques: it only takes 2 h, including sample preparation, distillation and capillary GC analysis.

The results of macroscale analysis by classical techniques were found to be identical with those obtained with the microapparatus described here. Some applications are given to illustrate this technique.

EXPERIMENTAL

Microdistillation apparatus (Fig. 1) and distillation

The plant material (0.2–3 g) is suspended in 50 ml of distilled water in flask A, electrically heated to boiling and distilled for 30 min. The essential oil is collected in bubble B in 100 μl of a light solvent (pentane or hexane) to avoid losses through emulsion formation. Water is continously recycled in flask A through arm C. The system is cooled by forced circulation of water, which is cooled to 2–3°C by a refrigeration system. Rigorous standardization of the operating conditions must be observed and analytical-reagent grade solvents must be used.

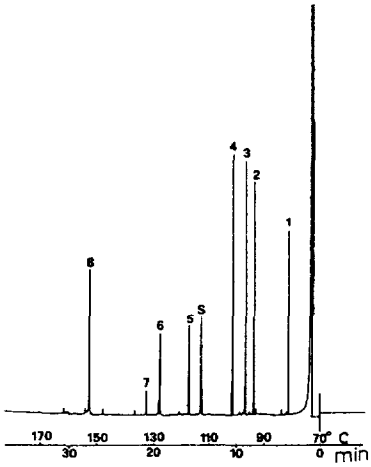


Fig. 2. Gas chromatogram of the test mixture after distillation. For peaks see Table I.

Capillary gas chromatography

Capillary GC analyses were performed on an SE-30 glass capillary column (35 m × 0.3 mm I.D.; film thickness, d_f = 0.3 μm) installed in a Carlo Erba 2900 gas chromatograph. The operating conditions were as follows: split injection system, 1:100, injection temperature, 250°C; column temperature, programmed from 70 to 190°C at 3°C min^{-1}; detector, flame ionization at 300°C; carrier gas, hydrogen at a flow-rate of 2 ml min^{-1}.

Peak areas were measured by means of a Perkin-Elmer Sigma 10 chromatography data station.

Plant material

The plant material was kindly supplied by Professor Tommaso Sacco of the Botanical Garden of Turin University and by Silvio Stefenelli of the Botanical Garden "Paradisia", Valnontey, Cogne (Italy).

TABLE I

RECOVERY OF THE METHOD FOR TEST COMPOUNDS

Peak No.	*Compound*	*Recovery (%)*
1	α-Pinene	93
2	Linalool	96
3	Camphor	101
4	Menthol	96
S	Ethyl decanoate	–
5	Carvacrol	85
6	Geranyl acetate	91
7	β-Caryophyllene	90
8	Viridiflorol	92

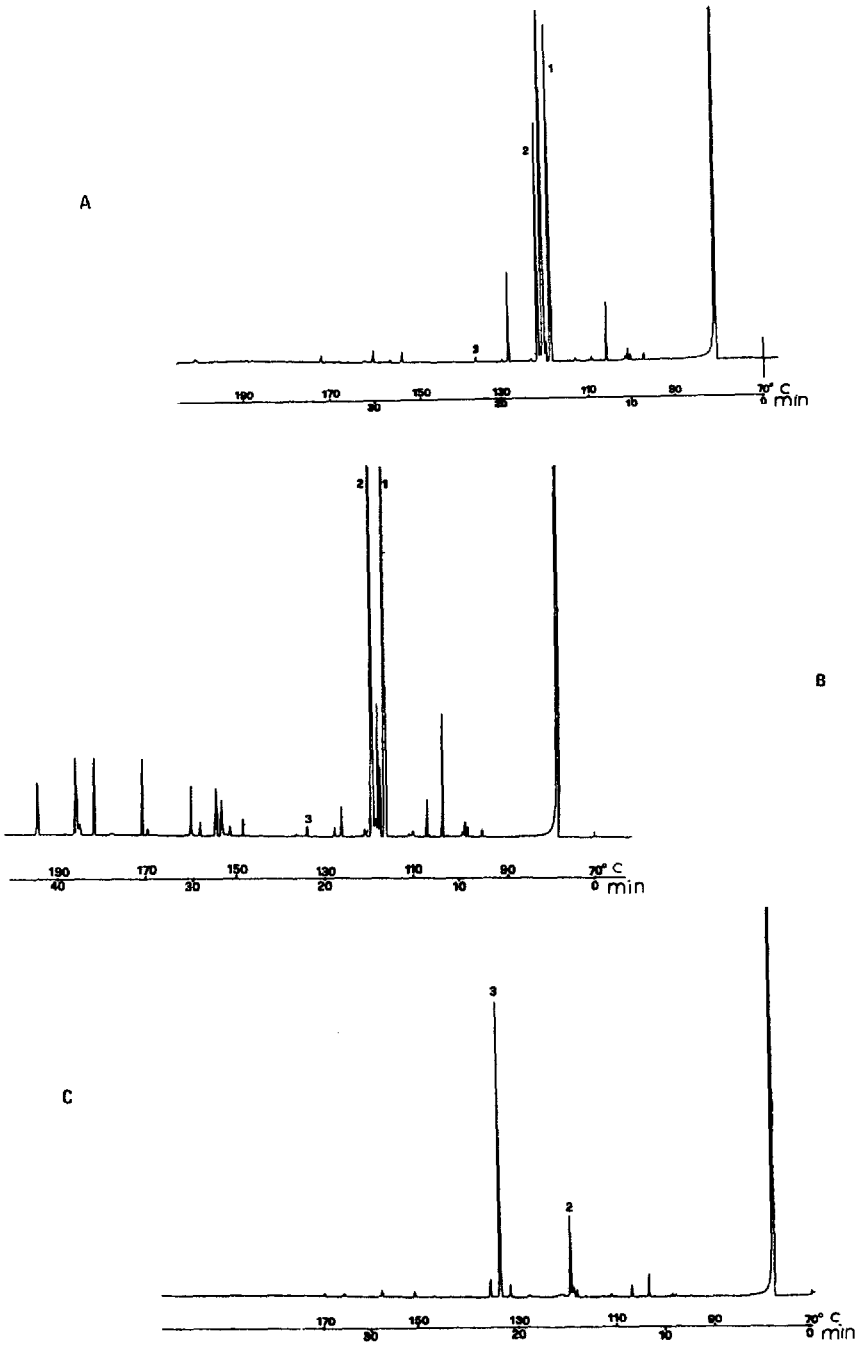


Fig. 3. Gas chromatogram of the essential oil of *Mentha piperita* L. A, Inflorescence; B, apical leaves; C, basal leaves. Peaks: 1 = menthone; 2 = menthol; 3 = menthyl acetate.

Test mixture

The components of the test mixture were α-pinene, linalool, camphor, menthol, carvacrol, geranyl acetate, β-caryophyllene and viridiflorol. The mixture, dissolved in 1 ml of pentane to which had been added 50 μg of ethyl decanoate as an internal standard, was analysed by capillary GC. A 100-μl volume of the test mixture was distilled in the micro apparatus, as described for plant material. The 100 μl of pentane in bubble B (see Fig. 1) contained 5 μg of ethyl decanoate as an internal standard. The distillate was analysed by capillary GC.

RESULTS AND DISCUSSION

Recovery

The quantitative aspects of recovery were tested with a test mixture (see Experimental). The mixture consisted of eight compounds, widely distributed among essential oils and presenting different functional groups.

Fig. 2 shows the gas chromatogram of the test mixture after distillation. The recovery of the individual components is given in Table I.

The sample size is obviously a function of several parameters, primarily the percentage of essential oil in the plant under investigation, and then the number of significant constituents present in the oil and the injection technique used. As a general rule, when the split injection system (1:100) is used and the oil contains ten main components (more than 2% each), 0.5 mg in 100 μl of solvent is ample for a significant gas chromatogram. Consequently, for a plant with 0.1% of essential oil, 0.5 g of fresh plant material is sufficient. Lower limits of sensitivity can be obtained by using a cold on-column injection system. The apparatus can be routinely operated with sample sizes between 0.2 and 4 g of plant material.

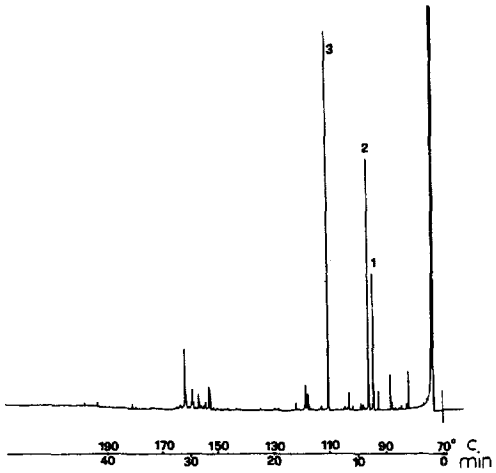


Fig. 4. Gas chromatogram of the essential oil of *Tanacetum vulg.* L. Peaks: 1 = α-thujone; 2 = β-thujone; 3 = chrysanthenyl acetate.

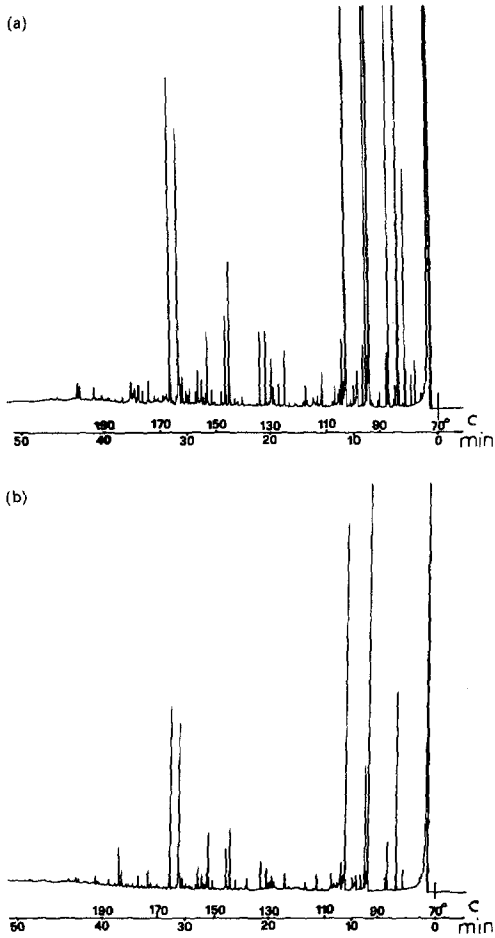


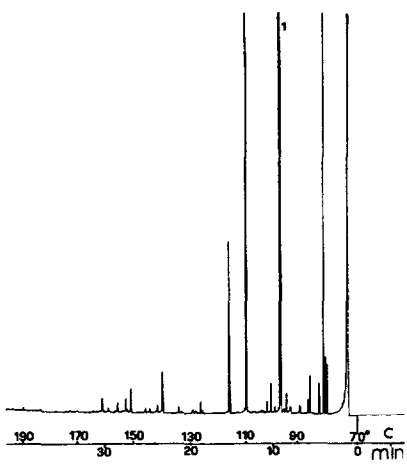
Fig. 5. Gas chromatograms of the essential oils of (a) wild-growing and (b) cultivated *Artemisia genipi* Weber.

Applications

Six different applications of the technique are reported for which classical methods do not give acceptable results.

Study of the composition of the essential oil of different parts of a plant. The composition of *Mentha piperita* L. essential oil in the basal leaves (fresh sample size 0.8 g), the apical leaves (0.8 g) and the inflorescence (0.2 g) from a single plant was investigated and showed considerable variation, as was expected (Fig. 3). The basal older leaves, for instance, show the presence of considerable amounts of menthyl acetate, which is presumed to be the end product of the metabolic pathway of the plant (menthone→menthol→menthyl acetate), while the apical leaves are richer in menthone.

Differentiation between mixed growth of two pure chemotypes of a plant species in a restricted area and the existence of a hybrid of the two. Tanacetum vulgare L. [*Chrysanthemum vulgare* (L.) Bernh.] is a plant widely distributed throughout Europe,



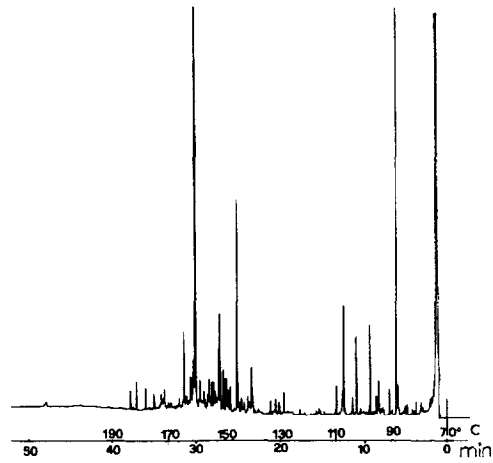


Fig. 6. Gas chromatogram of the essential oil of *Chrysanthemum parthenium* Bernh. Peak 1 = β-thujone.

Fig. 7. Gas chromatogram of the essential oil of *Artemisia chamaemelifolia* Vill.

and many chemotypes of it are known. In some instances the distillation of groups of tansy plants growing in a very restricted area (1–2 m^2), distilled together, gave an essential oil with high percentages of both thujone and chrysanthenyl acetate: these are two characteristic compounds defining two different chemotypes of *Tanacetum vulgare* L. The doubt was whether we were dealing with an actual chemotype (mixed thujone–chrysanthenyl acetate) or with two different plant chemotypes growing by chance close together. The distillation of individual plants, taken from the same area one by one, resolved this doubt in favour of the hybrid (Fig. 4).

Control of suitability for cultivation of plants of high economic interest, based on the essential oil as a control parameter. The suitability for cultivation of *Artemisia genipi* Weber[4], a rare and very expensive plant widely used in the liqueur industry in the Aosta Valley, was demonstrated by comparing the composition of the essential oils of cultivated and wild-growing plants. In fact, the two oils are pratically identical, as can be seen from Fig. 5. With the microapparatus described here, the control analyses were carried out on very small amounts of this expensive plant material (0.2 g).

Control of the influence of botanical degeneration on the composition of the essential oil. In the cultivation of *Artemisia genipi* Weber, some plants were found to be degenerated from a botanical point of view. The analysis of the essential oils of these forms (0.2 g) proved that this degeneration did not influence their composition, which was identical with that reported in Fig. 5.

First screening on an essential oil of unknown composition of a plant available in very small amounts. This application, of course, can be of very general use. The example reported here concerns the essential oil of *Chrysanthemum parthenium* Bernh. A single plant of *Chrysanthemum parthenium* Bernh. was found in an area where it had never been found before. With the microdistillation apparatus it was possible to show that the composition of the essential oil of this single plant was identical with that reported for other samples, growing in areas where this plant is very common. The gas chromatogram is shown in Fig. 6.

Study of plants containing small amounts of essential oil. Artemisia chamaemelifolia Vill. is a plant growing in the Alps at more than 1500 m above sea level, which possesses an intense smell, but contains low concentrations of essential oil (less than 0.3%). Fig. 7 shows the gas chromatogram of its essential oil.

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